Technical and Bibliographic Notes / Notes techniques et bibliographiques

The Institute has attempted to obtain the best original copy available for filming. Features of this copy which may be bibliographically unique, which may alter any of the images in the reproduction, or which may significantly change the usual method of filming, are checked below.

L'Institut a microfilmé le meilleur exemplaire qu'il lui a été possible de se procurer. Les détails de cet exemplaire qui sont peut-être uniques du point de vue bibliographique, qui peuvent modifier une image reproduite, ou qui peuvent exiger une modification dans la méthode normale de filmage sont indiqués ci-dessous.

☑ Coloured covers/
Couverture de couleur

☐ Covers damaged/
Couverture endommagée

☐ Covers restored and/or laminated/
Couverture restaurée et/ou pelliculée

☐ Cover title missing/
Le titre de couverture manque

☐ Coloured maps/
Cartes géographiques en couleur

☐ Coloured ink (i.e. other than blue or black)/
Encre de couleur (i.e. autre que bleue ou noire)

☐ Coloured plates and/or illustrations/
Planches et/ou illustrations en couleur

☑ Bo[illegible]her material/
Re[illegible]tres documents

☑ Tight binding may cause shadows or distortion along interior margin/
La reliure serrée peut causer de l'ombre ou de la distorsion le long de la marge intérieure

☐ Blank leaves added during restoration may appear within the text. Whenever possible, these have been omitted from filming/
Il se peut que certaines pages blanches ajoutées lors d'une restauration apparaissent dans le texte, mais, lorsque cela était possible, ces pages n'ont pas été filmées.

☐ Coloured pages/
Pages de couleur

☐ Pages damaged/
Pages endommagées

☐ Pages restored and/or laminated/
Pages restaurées et/ou pelliculées

☑ Pages discoloured, stained or foxed/
Pages décolorées, tachetées ou piquées

☐ Pages detached/
Pages détachées

☑ Showthrough/
Transparence

☐ Quality of print varies/
Qualité inégale de l'impression

☐ Continuous pagination/
Pagination continue

☐ Includes index(es)/
Comprend un (des) index

Title on header taken from:/
Le titre de l'en-tête provient:

☐ Title page of issue/
Page de titre de la livraison

☐ Caption of issue/
Titre de départ de la livraison

☐ Masthead/
Générique (périodiques) de la livraison

☐ Additional comments:/
Commentaires supplémentaires:

This item is filmed at the reduction ratio checked below/
Ce document est filmé au taux de réduction indiqué ci-dessous.

10X				14X				18X				22X				26X				30X		
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	✓	☐	☐	☐	☐	☐	☐	☐
		12X				16X				20X				24X				28X				32X

UNIVERSITY OF TORONTO STUDIES

PHYSIOLOGICAL SERIES

No. 23: **THE DIAGNOSIS OF ACIDOSIS,** BY PROFESSOR J. J. R. MACLEOD

(REPRINTED FROM THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE, VOL. IV)

THE UNIVERSITY LIBRARY: PUBLISHED BY
THE LIBRARIAN, 1919

UNIVERSITY OF TORONTO STUDIES
COMMITTEE OF MANAGEMENT

Chairman: SIR ROBERT ALEXANDER FALCONER, LL.D., K.C.M.G.
President of the University

PROFESSOR W. J. ALEXANDER, PH.D.
PROFESSOR W. H. ELLIS, M.A., M.B.
PROFESSOR J. J. MACKENZIE, B.A., M.B.
PROFESSOR J. P. MCMURRICH, PH.D.
PROFESSOR G. H. NEEDLER, PH.D.
PROFESSOR GEORGE M. WRONG, M.A.

General Editor: H. H. LANGTON, M.A.
Librarian of the University

THE DIAGNOSIS OF ACIDOSIS

A Review and Criticism of the Methods
at Present in Use

BY

J. J. R. MACLEOD, M.B.,
Toronto, Canada

Reprint from
THE JOURNAL OF
LABORATORY AND CLINICAL MEDICINE
St. Louis

Vol. IV, No. 6, March, 1919

THE DIAGNOSIS OF ACIDOSIS*

A REVIEW AND CRITICISM OF THE METHODS AT PRESENT IN USE

BY J. J. R. MACLEOD, M.B., TORONTO, CANADA

THERE has been some confusion in medical literature concerning the exact meaning of the term "acidosis," so that it will be advantageous to preface our discussion of the subject with a brief review of the work upon which the present-day definition is based. The discoveries that large quantities of oxybutyric and oxyacetic acids are excreted in the urine of patients suffering from diabetic coma, and that there is a general similarity between the symptoms of this condition and those which follow the intravenous injection of strong acids (acid intoxication) in laboratory animals, were primarily responsible for the adoption of the term. In seeking earlier signs of acidosis than the actual symptoms of coma, however, examination of the urine for oxybutyric and acetoacetic acids or their oxidation product, acetone, was found to be of uncertain value, since these substances might also appear in decidedly large amounts in the urine of nondiabetic individuals. During starvation, for example, either complete or involving carbohydrate foods alone, acetonuria was frequently met with, which made it clear that the excretion of acetone bodies could not in itself be taken as a reliable indication of impending acidosis.

It was attempted therefore, to develop methods of diagnosis depending, not on the detection of the particular acids, but on the effects which might be produced by the accumulation in the organism of acids in general.

In the first place it was natural to expect that the blood would become less alkaline as the result of the acid production, and attempts were therefore made to measure the alkalinity of the blood by titrating with standard acid until the point of neutrality was reached, as judged by the change of tint of some indicator. It was hoped that the values obtained by this method would indicate in the blood of cases threatened with acidosis, the presence of more acids than in normal blood; that is, that a smaller amount of standard acid would require to be added to the former, than to the latter blood, in order to cause the tint of some indicator to change. Although with certain modifications this method is theoretically sound, it was found to have little practical value, partly because it requires large amounts of blood, and partly because the coloring matter has to be removed before the test can be applied. Moreover, it came to be recognized that the amount of added acid, with the indicators ordinarily used, would represent, not only the basic constituents that combine with acids in the quantities in which these could be produced in the organism, even under abnormal conditions, but also those, like proteins and phosphates, that might be called upon to functionate as bases when the limits of acidity compatible with life were greatly overstepped.

---

*Delivered before the Medical Section, Academy of Medicine, University of Toronto, Toronto, Canada.

It became necessary to seek for some other type of reaction which acids might set up in blood, and the well-known effect which these have in expelling carbon dioxide from its combinations with alkalies was investigated (Walter). It was found by experiments on animals that a marked diminution in the CO_2 content of blood was induced by intravenous injections of acids, and the same was observed to be the case in the blood of patients in diabetic coma (Naunyn).[1] In the light of more modern research, by which, as we shall see later, the delicacy and accuracy of this method is fully justified, it is somewhat surprising that it did not receive more extensive application. One serious difficulty stood in the way, namely, the technic of the estimation.

The next step depended on the discoveries of Haldane and Priestley[2] that the percentage of CO_2 in the alveolar air of normal individuals at atmospheric pressure is remarkably constant, and of Krogh that the amount of free CO_2 in the arterial blood is very nearly the same as that in the alveolar air. These facts led Haldane and his collaborators to formulate the hypothesis that the alveolar CO_2 must be proportional to the relative amounts of carbonic and of other (fixed) acids in the blood, and that when the latter are increased there must be a compensatory decrease in the former, provided the control of the respiratory function is normal. The first application of this principle on pathologic acidosis was made by Beddard, Pembrey and Spriggs,[3] who examined the alveolar CO_2 in diabetic patients, with the result that a very pronounced diminution was found whenever the comatous condition existed or was threatened. The rationale of this method and its limitations we shall discuss later; for the present it may be stated that the alveolar CO_2 may also be depressed in other diseases, particularly nephritis, as well as in normal persons whenever acids accumulate in the organism as a result of deficient oxidation in the tissues (Haldane, etc.).

Clearly, therefore, the term acidosis must not be confined to cases of diabetic coma. On the other hand the common appearance of acidosis in this disease, coupled with the fact that in it the acids are of a type that is different from that found in nephritis, or during partial asphyxiation, makes it important that some term should be set aside to designate the diabetic condition. Since the acids are closely related to ketones (acetones) in chemical structure, the term "ketosis" has been suggested, and is gradually coming into general use. Ketosis, therefore, means the form of acidosis that is caused by the appearance of ketonic acids in the organism. Other forms of acidosis have not as yet been dignified by special names.

The remarkably rapid development of our knowledge of this subject during recent years, coupled with its dependence upon certain fundamental principles of physical chemistry, has made it very difficult for those of the medical profession who have been out of college for several years to appreciate the full significance and value of closely observing the neutrality regulation in the animal body in order that incipient states of acidosis may be detected. I will therefore attempt to review very briefly these fundamental principles, and to explain the various tests by which alterations in the reaction of fluids in general may be detected.

In the first place we must clearly understand what is meant by an acid such as HCl, and an alkali, or base, such as NaOH. When the molecule of either of these chemicals is dissolved in water it splits, or dissociates, into two portions called ions; in the case of HCl, for example, ions of H and Cl are formed, and in the case of NaOH, Na and OH. Moreover, each of the ions carries an electric charge, and this is always of opposite sign for the two ions composing the molecule. The sign of the electric charge for the H ion and all metals is positive, and that of Cl and all acid groups is negative. Since, as is well known, unlike electricities attract each other, this means that the positive ions like H would be attracted towards the negative pole or cathode of a pair of electrical terminals, between which a current is passing, placed in the solution. They are, therefore, called cations, and for the same reason the negative ions are called anions. To indicate these electrical charges, a dot is used for positive, and a dash for negative, thus, $H^{\cdot}$ and Cl'.

But what, it may be asked, have these principles of electrolysis to do with the question of reaction? The answer is that it is the presence of free $H^{\cdot}$ ions that determines the acidity of a solution. If we examine the formula of all known acids it will be seen that they have hydrogen in some displaceable or dissociable form in the molecule, and, furthermore, if we measure the acidity, as judged by the common standards of this property, such as the ability to dissolve metals, the acid taste, the power of inverting sugars and starches, and so forth, we shall find that it runs parallel with the dissociability of the H ion from the rest of the acid molecule. The $H^{\cdot}$ ions present in a free state in the solution must, therefore, be an accurate measure of its acidity. This leads us to expect that the presence of free OH' ions must be a measure of alkalinity, since we know that when an acid and a base are brought together, the one neutralizes the other by a reaction which consists essentially in the combination $H^{\cdot}$ and OH' ions to form H_2O. This removes the ions from the free state and so locks them up, because molecules of water practically do not dissociate.

We have learned two fundamental principles, namely, that the standard of perfect neutrality must be where $H^{\cdot}$ and OH' ions exactly balance each other, and that the true acidity of a solution will be represented by the excess of free $H^{\cdot}$ ions over OH' ions, that is, by the $H^{\cdot}$-ion concentration (C_H).

But we can go further, for not only may the acidity be expressed in terms of C_H, but so also may the alkalinity. Why should this be? It is clear that the most strictly neutral solution must be pure water, in which $H^{\cdot}$ and OH' ions are exactly counterbalanced. Nearly all of the $H^{\cdot}$ and OH' are combined in an undissociated molecule H_2O, but not all, for even in the purest water a slight degree of dissociation occurs, giving us therefore $H^{\cdot}$ and OH' ions. When the concentrations of the two ions are multiplied together the product is $1.2 \cdot 10^{-14}$, which means that there are 1.2 gram molecules of hydrogen (or its equivalent) present in, 10,000,000,000,000 liters. Since the concentrations of $H^{\cdot}$ and OH' ions are equal, the $H^{\cdot}$ ion must therefore, be $1.2 \cdot 10^{-7}$, which means that this ion is present so as to form a .000,000,12 N solution is 1.2 gm. $H^{\cdot}$ in 10,000,000 liters.

When some acid is added to the water the con. of $H^{\cdot}$ ions, of course, rises, but, and this is the fundamental point to bear in mind, the con. of OH' ions correspondingly falls so that, as in pure water, *the product of the two concentrations is again* $1.2 \cdot 10^{-14}$. However acid or alkaline a solution may be, the product of the concentrations of the $H^{\cdot}$ and OH' ions is always the same. Clearly then *we may express the reaction even of alkaline solutions in terms of the H ion concentration.* Whenever this is greater than $1.2 \cdot 10^{-7}$, the reaction is acid, but when it is less than $1.2 \cdot 10^{-7}$, the reaction is alkaline.* It is usual to abbreviate the expression hydrogen-ion concentration into C_H.†

These considerations lead us to seek for methods by which C_H may be measured. These are two in number, namely, the electrical and colorimetric. Concerning the former, suffice it to say that it consists in measuring the voltage or electric force set up in a battery of which one electrode is pure hydrogen gas in intimate contact with the solution whose C_H we desire to measure, and the other electrode is one of known voltage, such as the so-called calomel electrode. The rate of diffusion between the free $H^{\cdot}$ ions in the solution and the H which constitutes the one electrode, is naturally dependent upon the concentration of free $H^{\cdot}$ ions, and it is on this that the development of electric force depends at this electrode. Consequently since everything else is constant in the battery, the total electromotive force must be proportional to the C_H of the unknown solution.

The colorimetric method is much simpler, but not so delicate. It depends on the fact that certain indicators change in tint in proportion to C_H. In titrating solutions, such as the stomach contents, for the degree of acidity, the impression is apt to be formed that the tint changes suddenly at the neutral point. This is not the case however, for if the titration be done by very small additions of acid or alkali about the neutral point, it will be found that there is a fine gradation from the typical acid color to the typical alkaline color.

---

*The exact value of 1.2 varies with temperature. When C_H becomes greater than that of neutrality the number 1.2 becomes greater, and if the change be beyond 10 the characteristic (-7) becomes less.

†For convenience of expression it is usual to designate the H ion concentration by P_H instead of C_H. P_H is obtained by finding the logarithm of the number of gram molecules of H (i.e., 1.2 in the above example), and subtracting this from the characteristic (i.e., -7 in the above example). Since the minus sign is understood P_H increases in magnitude as the H ion concentration (expressed by C_H) decreases. In the present article C_H is used because its use is less confusing in a general discussion of the acidosis problem.

The exact C_H at which indicators change in tint varies with the indicator. Thus phenolphthalein, which is used in certain titrations of stomach contents, changes in tint at a much lower C_H than methyl orange or litmus, and with none of these indicators does the point of change occur at a C_H of 1.2×10^{-7}, so that they are unsuitable for measurement of the C_H of solutions that are nearly neutral. Fortunately, however, such indicators exist, the best known of which are sulphophenolphthalein, rosolic acid, and neutral red. If therefore we take a series of solutions having slightly variable, but known, C_H about the neutral point, and add to each a drop of one of the latter indicators, we shall obtain a series of graded tints, and the tint will be proportional to the C_H. The preparation of the standard solutions is not a difficult matter, provided ordinary precautions are taken. Mixtures of acid and basic phosphates in varying proportions are most practical.[4] The resulting mixtures should be measured electrometrically for accurate work. In order to determine the C_H of an unknown solution, some of it is placed in a hand glass test tube and an amount of suitable indicator is added so that the proportion of indicator to solution is the same as in the standards. The tint of the standards with which the tint of the unknown matches is then ascertained and this gives C_H. When the colorimetric method is employed for blood, it is of course necessary to get rid of the blood pigment and also of the proteins since these interfere with the reaction. This is accomplished by placing a few cubic centimeters of blood in a dialyser in the shape of a collodion tube, and suspending this in neutral physiologic saline. The saline soon assumes the same C_H as the blood, and this can be measured by the above described method.

When C_H of the blood is measured by one or other of these methods the significant fact is revealed that it is practically always the same, and is not far removed from that of pure water; at 38° C. C_H equals 0.4×10^{-7}. Even in severe cases of diabetic ketosis, an increase in C_H becomes perceptible only in the final stages of the condition. Clearly, therefore, even the slightest increase in C_H is incompatible with life, and C_H of the blood must be considered as a physiologic constant. It is greatly more so than body temperature or blood pressure, so that its measurement can be of little practical value in the clinic, and the question arises as to how the early stages of changes that might ultimately end in death from an increase in C_H may be detected. To answer this question we must study *the nature of the mechanism by which neutrality is maintained in the organism;* in other words why it should be the case that large quantities of acid can be produced in the body, as in diabetes, without any perceptible change in C_H.

When even a trace of acid is added to water or an isotonic solution of sodium chloride, a very pronounced change occurs in C_H, but it requires ever so much more acid to produce any perceptible change in the case of blood. For example, using the colorimetric method let us observe the change in tint produced by adding a drop of weak HCl to water containing sulphophenolphthalein and then see how much of the same acid must be added to bring about a similar change of tint in a dialysate of blood. It takes very much more. Clearly the blood contains something which as it were soaks up the added H ions. This has been called the ***buffer action*** of blood, or better still, the ***tampon action***. The question is, to what is this buffer action due? A clue is furnished by using solutions of phosphates. For example, if we take a solution containing alkaline and acid phosphates in such proportion that C_H is the same as that of blood, and then add acid, we shall find that ever so much more has to be added than is the case of water. The same is true of solutions of bicarbonate.

This property of phosphate has been carefully studied by L. J. Henderson,[5] to whose brilliant researches we are primarily indebted for the recent development of our knowledge in this whole question. Henderson found that weak acids like acid phosphate and carbonic acid possess the property of holding the H-ion concentration nearly constant, i.e., of acting as buffers, when they are present in solutions containing an excess of their salts. Now, in so far as the blood plasma is concerned, it is not phosphates, but rather bicarbonates to which the buffer action must be due, and as a matter of fact it has been found that C_H is directly proportional to the ratio existing between CO_2 in solution as H_2CO_3 and sodium bicarbonate $NaHCO_3$ multiplied by a constant; or, expressed in chemical notation, C_H = the molecular ratio $\frac{H_2CO_3}{NaHCO_3}$. This ratio is $1/20$, and we may define acidosis as any condition in which the proportion between H_2CO_3 and $NaHCO_3$ becomes greater than 1:20. It must be clearly understood that this applies only to isolated plasma, for when whole blood is used, other substances come into play in maintaining neutrality; the phosphates, for example, though practically absent from plasma, are nevertheless present in the corpuscles through the envelopes of which diffusion more or less readily occurs, so that they serve as a reserve buffer. Proteins also may serve either as weak acids or alkalies, and therefore neutralize quite decided quantities of added acid or alkalies. But for practical purposes we may regard the buffer agency as being the ratio $\frac{H_2CO_3}{NaHCO_3}$ and the problem of finding practical means for the detection of threatened acidosis now narrows itself down to a study of the behavior of this ratio.

Suppose that some fixed acid were added to a buffer solution containing bicarbonate. It would react with $NaHCO_3$ to form a neutral salt of the acid, and consequently set free some H_2CO_3, that is, it would diminish the denominator but increase the numerator of the equation $1/20$. The increase in C_H would not be proportional to that of the added acid, on account of the buffer mechanism (because H_2CO_3 does not dissociate well) but it would nevertheless increase somewhat just as any kind of buffer takes up most, but not all, of a force applied to it. This indicates that if the foreign acid were being added continuously, as would be more or less the case in pathologic acidosis, C_H would rise in spite of the buffer unless some method existed for decreasing the numerator of the equation, i.e., getting rid of H_2CO_3. This is one of the functions of the lungs, the CO_2 excretion through which must therefore be considered as a necessary part of the neutrality mechanism of the body. The CO_2 is got rid of until the ratio $\frac{H_2CO_3}{NaHCO_3}$ comes back to its old level of $\frac{1}{20}$. But now, much of the $NaHCO_3$ having been used up to combine with the foreign acid, the actual amount present is much less than before. This means that the H_2CO_3 must also be less, that is, the CO_2 in a free state in the blood plasma. Now, since as we have seen, the pulmonary epithelium permits the free CO_2 of the blood to diffuse readily through it, it follows *that the percentage of CO_2 in the alveolar air must be a measure of the available $NaHCO_3$ in the blood.* To repeat, too, this is the fundamental conception of the whole acidosis problem, since C_H remains constant in the blood the ratio $\frac{H_2CO_3}{NaHCO_3}$ must also remain at its normal value of $\frac{1}{20}$, and, therefore, if $NaHCO_3$ declines, H_2CO_3 must decline proportionately, and since this diffuses as CO_2 into the alveolar air, the percentage of this gas in the latter must be proportional to the degree to which foreign acid can be added to the blood without perceptibly changing C_H, in other words it must be proportional to the reserve alkalinity.

One other factor must clearly come into play to permit of the smooth operation of the above mechanism, namely, the rate of pulmonary ventilation must be adapted to the amount of CO_2 that has to be eliminated. This adaptation depends on the respiratory center, the activity of which is preeminently dependent upon the acid base equilibrium in the blood.

It is commonly taught that the thing that really stimulates the respiratory center is not the CO_2 itself, but the slight increase in C_H which, as explained above, inevitably occurs, in spite of the buffer action. This is, however, probably an incorrect view, for R. W. Scott,[6] working in my laboratory, has found that CO_2 itself can excite the center quite independently of the H-ion concentration of the blood. Thus Scott found after injecting sodium carbonate into decerebrate cats until the C_H was very decidedly lowered, that a subsequent increase in the free CO_2 of the blood excited respiration almost to the same degree as would have been the case when the C_H of the blood was the normal, and furthermore, that when this excitement occurred, the blood was still markedly alkaline.

So long then as the center responds immediately to the slight excess of free CO_2 this is got rid of, and the normal ratio between H_2CO_3 and $NaHCO_3$ is reestablished. If the sensitivity of the center should be below par, however, then CO_2 might accumulate in the body and C_H consequently rise. As a matter of fact, even in health, it seems to be established that the excitability of the center may vary, as for example, in relationship to the amount of oxygen in the blood supplying it, and in disease there can be no doubt that such variations in excitability occur. And since we know that the activities of this center are also greatly affected by nerve stimuli, arriving at it along afferent nerves, it is to be expected that it may be keyed up or down in excitability by the state of the nervous system in general. In conditions of nervous excitement and anxiety, for example, its sensitivity is increased and moderate doses of narcotic drugs, such as morphine, are well known to depress it.

But even were the sensitivity of the center maintained at a constant level, the alveolar-CO_2 would correspond with that of the arterial blood only provided that the exchange of CO_2 across the alveolar epithelium was strictly normal. And when we bear in mind that the proper excretion of the CO_2 in the lungs is dependent upon an accurate adjustment between the heart's action and the rate of CO_2 production in the organism, we see how the mechanism might readily become upset.

Technical difficulties have also to be overcome in the collecting of the alveolar air, for it is now well established that the original method of Haldane and Priestley is approximately accurate only when it is carried out under strictly controlled conditions—so strict that they can not be practiced in the clinic—and even then, as R. G. Pearce, Carter, Krogh, Siebeck and others[7] have shown, we can not be certain of the results. At best, therefore, *the alveolar CO_2 can serve as an accurate index of the acid base equilibrium of the blood only under certain controlled conditions.*

These facts have prompted the most recent observers (Morawitz and Walker[8] and later Van Slyke and Cullen[9]) to return to blood examination for the detection of impending acidosis. The question is what readily measurable property of the blood may we employ? If we return for a moment to the equation $\frac{H_2CO_3}{NaHCO_3} = \frac{1}{20}$ we shall see that when foreign acids combine with the Na of the bicarbonate, this will become replaced by the salts that are formed, such as NaCl, and these will be incapable of acting as buffers. The amount of $NaHCO_3$ present in the blood must, therefore, be a measure of its power to take up such acids without serious disturbance in C_H, which has led Van Slyke to define acidosis as "a condition in which the concentration of bicarbonate in the blood is reduced below the normal level." According to this definition, acidosis is not necessarily an increase in the actual C_H of the blood, or even it is a disturbance in the normal ratio $\frac{H_2CO_3}{NaCO} = \frac{1}{20}$ but is a lowering of the absolute values forming the numerator and denominator of the equation. In brief, it is a lowering of the ability of the blood to take up fixed acid without disturbance in the ratio, a decrease in the reserve alkalinity. The definition is, however, somewhat unfortunate, since it does not include cases in which there is an actual increase in the ratio as a result of the addition of H_2CO_3 to the blood, and such conditions may develop as a result of asphyxia, which, of course, is a common enough cause of acidosis in the broader sense. Moreover, in asphyxial acidosis it may quite well be the case that the $NaHCO_3$ instead of being diminished, is actually increased in the attempt to bring the ratio back to its normal value. When CO_2 is added to blood, for example, it has been shown that the alkali content of the plasma relatively increases, partly because of the migration of K and Na out of the corpuscles into the plasma, and partly because HCl goes in the opposite direction. There can be no doubt, however, that deficiency in bicarbonate is an important thing to measure as a gauge of the ability of the blood plasma to h[illegible] constant when foreign acids are added to it.

In its newer form this test differs considerably from the form in which it was originally employed by Walter (see p. 3), for instead of measuring the CO_2 content of a sample of blood immediately after removal, this is first of all exposed outside the body to an atmosphere containing a known, fixed, percentage of CO_2 until all of the available alkali has become combined with this gas. This procedure removes the most serious objection to the old technic, namely, that the amount of CO_2 in the venous blood,—and that alone is of course available for use in man,—must be very largely dependent upon the rate of oxidation in the tissues, and upon the velocity of movement of blood through the capillaries which drain into the vein. In experimental work on animals, however, the CO_2 actually present in arterial blood remains as the most practical indicator of the buffer action of the blood. Being restricted in man to the use of venous blood, the questions which remain to be answered are: 1. What pressure of CO_2 should be chosen with which to saturate the blood?* 2. Should whole blood or plasma or serum be employed? With regard to the CO_2 pressure, there are two alternatives: we may use either the pressure to which the blood is actually exposed in the organism, the *intra vitam* pressure we may call it, or an arbitrarily fixed pressure. The *intra vitam* pressure of CO_2 can be determined by analysis of the alveolar air, but this, of course, complicates the technic and gives us no more accurate an estimate of the available alkali than when we use the same pressure for all samples.

Concerning the question as to whether whole blood or serum should be used, there can be little doubt that it should be the former. The objection to the use of serum or plasma depends on the fact, as we have seen, that a part of the alkaline reserve resides, not in the fluid menstruum of the blood, but in the corpuscles, and even beyond this, in the tissue cells. It has been known for some time, for example, that when CO_2 is bubbled through whole blood, the alkali content of the plasma decidedly increases, as judged by titration, and when the blood is in contact with the tissue cells it is quite likely that when excess of acid appears in it, there may be a considerable transference, not only of more alkali from the cells into the blood, but also of CO_2 in the opposite direction, (into the cells including the red blood corpuscles) in which it may become combined with alkaline phosphates by such reactions as are illustrated in the equation:

$$Na_2HPO_4 + H_2CO_3 = NaH_2PO_4 + NaHCO_3.$$

It should be remarked that Van Slyke recognizes these possibilities, but nevertheless, he believes that for practical purposes it is allowable to employ venous blood, and to saturate its plasma with CO_2 at a definite partial pressure. The results which he and other workers using his method have employed would certainly appear to justify his claim at least for the detection of cases in which the alkaline reserve is decidedly reduced, but it should not be lost sight of that a strict observance of the principles which we have attempted to explain might make the gauging of the alkaline reserve a still more valuable criterion of disturbances in the acid base equilibrium in the organism.

---

* [illegible] may be pointed out, for the sake of those who are not familiar with the work in this field, that the pressure of a gas in a mixture of gases is proportional to its percentage amount, thus [illegible] cent CO_2 will give 45.6 mm. Hg pressure at a barometric pressure of 760, for 100 : 760

To sum up, it may be said that determination of the ability of blood to absorb CO_2 is probably the most practical method for measuring the acid neutralizing power; it measures the degree to which the acid buffer can functionate or, as some call it, the alkaline reserve. This high estimate of its value holds good, however, only when the whole blood is taken, but *even then we do not necessarily measure the total reserve of the body* These reserves are, first, the alkalies of the plasma, second, the alkalies of the corpuscles, third, the proteins of the blood, and the last reserves are probably the alkalies and the proteins of the tissue cells. Now it is clear that there can be no test-tube method by which the magnitude of all of these defensive agencies could be measured; at best we can only measure the first three of them, namely, the reserve power resident in the blood itself. Christiansen, Haldane and Douglas[10] and contemporaneously Morawitz and Walker,[8] have recommended this method, and it is only more recently that Van Slyke, in order to simplify the technic, has advocated the employment of blood plasma (oxalated) alone. Let us consider for a moment, therefore, whether the Van Slyke technic is really much simpler than that employed by Haldane. In the method used by these observers a cubic centimeter, or so, of defibrinated blood is exposed at body temperature for twenty minutes, in an air-tight vessel, to an atmosphere containing a known amount of CO_2. A measured sample is then removed to the gas analysis apparatus of Haldane-Barcroft, and the CO_2 is determined by decomposing the carbonates with strong acid, after getting rid of the oxygen by shaking with ferricyanide solution. The procedure is comparatively simple, and can easily be done with a little practice in the wards.

In Van Slyke's method, the oxalated plasma, separated by rapid centrifuging, is exposed to an atmosphere of expired air in a tonometer, and the plasma then transferred to a gas analysis apparatus, that is certainly no simpler in manipulation than that of Haldane and Barcroft (see Vol. II, p. 55 of this Journal), and which moreover suffers from the disadvantages: first, that the slightest leakages around the stopcock involves a very serious error because of the vacuum which is established in the apparatus at a certain stage of the manipulation, and secondly, that it requires the use of mercury which becomes fouled with the mixture of plasma and acid during the analysis.

Taking into consideration the theoretic objections to the use of plasma or serum in place of blood, and also the doubtful advantage to be gained by using the Van Slyke apparatus, at least by those who are not practiced in the use of gas analysis methods in general, there is no very evident reason why the Van Slyke procedure should be followed in preference to the earlier, really simpler methods of Haldane, etc., and of Morawitz and Walker.

It has been pointed out above that the sensitivity of the respiratory center towards the C_H, or more probably the free CO_2, of the blood is apparently much greater than that of any other center or mechanism in the animal body, and that it is on account of this sensitiveness that the free CO_2 of the blood is immediately got rid of from the body by increased pulmonary ventilation, whenever there is tendency for the C_H to rise above the normal level. As the free CO_2 is got rid of, the bicarbonate decomposes, because of the presence of other acid groups in the blood, e.g., protein, etc., and the amount that is left indicates the remaining ability of the blood to withstand further addition of foreign acid. Clearly, therefore, the important thing to measure in order that we may be enabled to diagnose the incipient stages of acidosis is the alkaline reserve, and but one question remains to be considered, namely, whether arterial or venous blood should be employed. For various reasons arterial blood is preferable. In the first place the percentage of CO_2 actually present in it is proportional to the alkaline reserve, so that it is unnecessary to expose the blood to an atmosphere containing CO_2 before measuring the CO_2 content, and in the second place, it represents the mixed blood of the body, and not that of only one locality, as is the case with blood removed from a peripheral vein. But in clinical practice venous blood only is available. If this is collected with the precaution that the muscles in the corresponding area have been at rest for some time it appears that there is practically no difference between the alkaline reserve of arterial and venous blood, but if there has been any muscular contraction, the venous blood will have a lower reserve than the arterial, because of the lactic acid thrown into it by the contraction. But even when we take the precaution of avoiding muscular action, it is probable that there is not a strict parallelism between the buffer action of arterial and venous blood as in cases in which the demands on the alkaline reserves are such that those of the tissues are being called on as well as those of the blood itself. The experiment of Van Slyke,[9a] in which he compared actual CO_2-containing powers of arterial and venous blood removed from anesthetized dogs, and found them to be very nearly the same, does not throw any light on this phase of the problem.

The chief criticism against the use of the CO_2 carrying power of blood or blood plasma, is therefore, that it tells little if anything concerning the acid absorbing powers of the tissues. Is there not, therefore, some test of the acid buffer which can be applied to the intact animal? One such we have already considered, namely, the percentage of CO_2 in alveolar air, and we have seen that it is not entirely satisfactory partly because of the technical difficulties in the collection of the sample of air for analysis, and partly because of possible variations in the sensitivity of the respiratory center. In order to place an estimate on the relative value of these methods comparisons have been made between the CO_2 tension of the alveolar air and the CO_2 absorbing power of the blood. This has been done both in normal and pathologic subjects. In normal subjects the comparisons have been made under conditions, such as the taking of food and during muscular exercise, in which slight alterations in the acid-base equilibrium are known to occur. Van Slyke, Stillman and Cullen[9b] found that the ratio $\frac{\text{plasma } CO_2}{\text{mm. alveolar } CO_2}$ varies from 1.27 to 1.80 in different resting individuals, there being apparently a characteristic ratio for each individual, and that the taking of food invariably raises the alveolar CO_2-combining power. This would seem to indicate that it must be the excitability of the respiratory center rather than the acid base equilibrium that becomes altered so as to cause variations in alveolar CO_2. The same authors, in conjunction with Fitz, working on the above relationship in patients suffering from diabetes and nephritis, found that in the former disease, under treatment, the alveolar CO_2 tension is often much too low in comparison with the blood bicarbonate. On the other hand the tension is always low in these cases when the bicarbonate content of the blood is low. In nephritis, however, the tension of alveolar CO_2 may be high although the blood CO_2 is low. Peters[12] has also examined these relationships, and has met with many instances where satisfactory parallelism did not exist. One serious criticism of this work is, however, that the method of Fridericia was employed for collecting the alveolar air.

Christiansen, Douglas and Haldane[10] did not find a relation to exist between the CO_2 absorbing power of the blood and the normal resting alveolar CO_2 in different healthy individuals, but after severe muscular exercise, the interesting discovery was made that marked reductions occurred both in the CO_2 absorbing power of the blood, and in the alveolar air, and they suggested "that corresponding differences will probably be discovered under various pathologic or compensatory conditions for acidosis."

In all these comparisons it is with the arterial CO_2 tension that the alveolar CO_2 is believed to run parallel, the air samples being removed from the alveoli under conditions which mean that the gaseous equilibrium must be between alveolar air and arterial blood. The question therefore arises whether more exact parallelism between alveolar CO_2 and blood CO_2 might not be obtained if the venous CO_2 tension were taken instead of the arterial. As shown by Christiansen, Douglas and Haldane, and later by Y. Henderson[11] and R. G. Pearce,[13] the alveolar venous CO_2 tension can be measured by the comparatively simple expedient of causing the patient to inspire an atmosphere containing a percentage of CO_2 above that which corresponds to the free CO_2 of venous blood, and then removing successive samples of the expiration which follows, and analysing each for CO_2. The portions expired at first will contain approximately the same percentage of CO_2 as that in the inspired air, but the percentages will progressively decline in the succeeding portions until at last they become constant at a level which must correspond to the venous CO_2 tension. In the method of Plesch for the collection of alveolar air, in which the person takes several breaths in and out of a rubber bag, the CO_2 percentage of the air in the bag must approximate more closely the CO_2 tension of the venous blood than that of the arterial. It is certainly important that the possibility of a parallelism between alveolar venous CO_2 tension and CO_2 combining power of the whole blood should be thoroughly investigated. On *a priori* grounds the parallelism is likely to be a much closer one than when the comparison is made between alveolar (arterial) CO_2 tension and CO_2 combining power of blood plasma.

By an application of these principles, therefore, it does not appear that there is a simple, thoroughly reliable method by which the buffer action of the body as a whole can be measured. An entirely different plan of attacking the problem must be thought of, namely, to observe the acid excretion by way of the urine.

When we were considering the general nature of the chemical reactions between the salts of the blood and added acid we saw that besides free CO_2 there would have to be eliminated from the body a considerable amount of acid salts or of free acids. In this connection the body must be considered as a whole and when foreign acid is added a corresponding amount must ultimately be eliminated, if the normal acid base equilibrium is to be reestablished, even although a sudden change in C_H is avoided by the buffer action. Most of this excess acid, as we have seen, is eliminated by the lungs as CO_2, but the remainder has to be got rid of by other pathways of excretion, particularly the kidneys. The excretion of the acid excess by this pathway occurs in three forms: *first* as acid salts, for example by conversion of Na_2HPO_4 into NaH_2PO_4, *second* as salts of ammonia and *thirdly* as free acid. Concerning the second of these, under normal conditions ammonia is a product of metabolism split off during the break down of amino acids, and then rendered innoxious, by combining with carbonic acid to form ammonium carbonate which is converted into urea, a strictly neutral substance. When excess of acids must be neutralized, however, some of the ammonia instead of combining with CO_2, may be diverted to neutralize the excess, this being particularly the case when ketonic acids appear, as in diabetes. The ammonia excretion itself is, however, no reliable indicator of the amount of free acid in the body, because there are other conditions such as derangement of the hepatic function which may influence it. In the toxic states of pregnancy, for example a large excretion of ammonia may occur without any indication of an acidosis. With regard to the acid which may be excreted in a free state, this occurs, for example, with β-oxybutyric acid, which is so weak in its acid properties, that 45 per cent of it may be excreted in this form.

With such a multiplicity of forms of excretion it is clear that *the only satisfactory method for measuring the acid excretion by the kidney must be one in which all of the above forms of excretion are included.* To measure the amount of free acids and acid salts the theoretically correct method would be to titrate the urine with standard alkali until its C_H, which is normally on the acid side of neutrality, was brought to the same level as that of blood. This can be done by using sulphophenolphthalein which, it will be remembered, changes tint at about the C_H of blood. For practical purposes, however, it has been found more convenient to use phenolphthalein, the neutral point of which is such that when urine just reacts neutral to it, the CO_2 combining power of the blood plasma as tested by Van Slyke's method, is at its maximum by 80 vols. per cent, and the ammonia excretion is zero. By titrating a sample of urine after adding neutral oxalate to it,—in order to precipitate substances which interfere with the sharpness of the end point—with $N/_{10}$ NaOH to the neutral point of phenolphthalein we measure the total of free acids and acid salts, and if we now add to this the amount of ammonia in the same urine, (readily measured by Folin's new permutit method), we obtain the *total urinary acid excretion.* It is said that a definite relationship exists between the CO_2 combining power of the plasma and the total acid excretion, a relationship, however, which can be made clear only when a rather arbitrary equation like that of Ambard is employed. It remains to be seen of what value the determination of this ratio may be in clinical diagnosis.

There remains possible another method for gauging the alkaline reserve, namely, to see *how much alkali can be added to the organism without causing the* *urine to assume an alkaline reaction.* When the alkaline reserve of the body is about normal, it is clear that very little alkali will suffice to have this effect. As a matter of fact, it has been found by Sellards[11] and by Palmer and Henderson[12] that only 5 grams a day, can be taken without making the urine alkaline. When the alkaline reserve is seriously depleted, however, large quantities of bicarbonate, even as much as 100 grams a day can be taken without making the urine alkaline. This test has been found of particular value in the diagnosis of acidosis accompanying certain forms of renal disease (chronic interstitial nephritis), which raises the question as to whether the retention may not be due to faulty elimination of the bicarbonate rather than to its retention in order that a deficient alkaline reserve may be corrected. It has not been a very simple matter to entirely disprove this possible explanation, and experiments of a variety of types have had to be devised in connection with the problem. One of them consists in determining the effect of a second dose of bicarbonate administered to an acidosis patient to whom a sufficient amount had previously been given to render the urine just alkaline. It has been found that a few grams now suffice, indicating, apparently, that the alkaline reserve must have been restored to its normal level. Even to this experiment the objection can be raised, however, that the large doses were retained because the threshold of the kidney for the excretion of bicarbonate was a very high one, and that the second, smaller administration just sufficed to overstep this threshold.

Sellards' careful work with this method seems quite clearly to establish its value, however, and for practical purposes *it is no doubt the best test of acidosis at present available in routine clinical work.* It has the important advantage, furthermore, of being simple and of requiring no elaborate apparatus.

Finally it may be advantageous to classify the possible causes which might lead to a want of stability in the C_H of the blood; that is, to threatened acidosis or alkalosis, not of acidosis in the narrow sense implied in Van Slyke's definition, but in the broader sense of any disturbance in the acid-base equilibrium.

In general a tendency to acidosis might be due to an increase in the numerator or decrease in the denominator of the molecular equation $\frac{H_2CO_3}{NaHCO_3} = \frac{1}{20}$, or to a proportionate decrease in both. In the latter case, there would be no actual change in C_H, but the alkaline buffer would be depleted so that the change would very readily set in when foreign acids were added. Furthermore, it should be understood that $NaHCO_3$ only stands as a symbol for all substances that might serve as alkaline reserves, for although this salt is no doubt the most important of these, the alkaline phosphates of the corpuscles, and the protein of the blood and tissues must also be considered. A tendency to alkalosis—which is no doubt extremely rare as a pathologic condition—would be due to changes of a reverse character. A theoretic classification of the conditions which might cause these changes is given:

Increase of C_H

Addition or accumulation of acid	Accumulation of CO_2 (asphyxial conditions). Incomplete oxidation of carbohydrate (lactic acid in muscular exercise). Defective oxidation of fat (ketosis). Renal insufficiency (nephritis). Decomposition of protein (as in acidosis of fever). Intestinal fermentation. Administration of acid (experimental).
Decrease of base	Diarrhea and hemorrhage, respectively (may explain acidosis in cholera and in certain forms of shock).

Decrease in C_H

Addition or accumulation of base	Ammonia (faulty metabolism of urea). Intestinal putrefaction (infantile conditions). Administration of alkalies (experimental).
Removal of acids	Excretion of CO_2 (excessive pulmonary ventilation, as in faulty ether administration). Excretion of acid urine.

BIBLIOGRAPHY

[1] Naunyn, B.: Der Diabetes Mellitus, Vienna, 1906.
[2] Haldane, J. B., and Priestly, J. P.: Jour. Physiol., 1905, xxxii, 227.
[3] Beddard, Pembrey and Spriggs: Jour. Physiol., 1904, xliv, 31; ibid., 1908, xxxix, 37; also Brit. Med. Jour., 1908, ii, 580.
[4] Levy, R. L., Marriott, W. McKin., and Rowntree, L. G.: Arch. Int. Med., 1915, xvi, 389.
Clark and Lubs: Jour. Bact., 1917, ii, 1 and 109.
[5] Henderson, L. J.: Am. Jour. Physiol., 1905-06, xv, 257; ibid., 1908, xxi, 169 and 427; Jour. Biol. Chem., 1909, ix, 403.
[6] Scott, R. W.: Am. Jour. Physiol., 1918, xlvii, 43.
[7] Pearce, R. G.: Am. Jour. Physiol. 1907, xliii, 73; ibid., xliv, 369.
Hoover, D. R.: ibid., 391.
Krogh, A., and Linhard, J.: Jour. Physiol., 1914, xlvii, 431.
Haldane, J.: Am. Jour. Physiol., 1915, xxxviii, 20.
Carter, E. P.: Jour. Exper. Med., 1914, xx, 81.
[8] Morawitz, P., and Walker, J. C.: Biochem. Ztschr., 1914, lx, 395.
[9] Van Slyke and Cullen: Jour. Biol. Chem., 1917, xxx, 289.
[9a] Van Slyke and Cullen: Loc. cit., p. 363.
[9b] Van Slyke, Stillman and Cullen: Jour. Biol. Chem., 1917, xxx, 401.
[10] Christiansen, J., Douglas, C. C., and Haldane, J. B.: Jour. Physiol., 1914, xlviii, 246.
[11] Henderson, Y.: Jour. Biol. Chem., 1917, xxxii, 325.
[12] Peters, J. P.: Am. Jour. Physiol., 1917, xliii, 113.
[13] Pearce, R. G.: Proc. Am. Phy. Soc., Am. Jour. Physiol., 1918, xlv, 550.
[14] Sellards, A. W.: Principles of Acidosis, etc., Harvard University Press, 1917.
[15] Henderson Y., and Palmer: Jour. Biol. Chem., 1912, xiii, 393; ibid., xiv, 81; ibid., xvii, 305; ibid., 1915, xxi, 37.

UNIVERSITY OF TORONTO STUDIES

PHYSIOLOGICAL SERIES

No. 1	The structure, micro-chemistry and development of nerve-cells, with special reference to their nuclein compounds, by F. H. SCOTT	0.50
No. 2	On the cytology of non-nucleated organisms, by A. B. MACALLUM	0.75
No. 3	Observations on blood pressure, by R. D. RUDOLF	0.75
No. 4	The chemistry of wheat gluten, by G. G. NASMITH	0.50
No. 5	The palaeochemistry of the ocean, by A. B. MACALLUM	0.25
No. 6	The absorption of fat in the intestine, by G. E. WILSON	0.50
No. 7	The distribution of fat, chlorides, phosphates, potassium and iron in striated muscle, by MAUD L. MENTEN	0.25
No. 8	Surface tension and vital phenomena, by A. B. MACALLUM	1.00
No. 9	On the distribution of potassium in renal cells, by C. P. BROWN	0.25
No. 10	On the probable nature of the substance promoting growth in young animals, by CASIMIR FUNK and A. BRUCE MACALLUM	0.25
No. 11	The comparative value of lard and butter in growth, by CASIMIR FUNK and A. BRUCE MACALLUM	0.25
No. 12	The action of yeast fractions on the growth of rats, by CASIMIR FUNK and A. BRUCE MACALLUM	0.25
No. 13	A new conception of the glomerular function, by T. G. BRODIE	1.00
	On changes in the glomeruli and tubules of the kidney accompanying activity, by T. G. BRODIE and J. J. MACKENZIE	
No. 14	Further observations on the differential action of adrenalin, by FRANK A. HARTMAN and LOIS MCPHEDRAN	0.50
No. 15	The mechanism for vasodilatation from adrenalin, by FRANK A. HARTMAN and LOIS MCPHEDRAN FRASER	
No. 16	Adrenalin vasodilator mechanisms in the cat at different ages, by FRANK A. HARTMAN and LESLIE G. KILBORN	0.25
No. 17	Location of the adrenalin vasodilator mechanisms, by FRANK A. HARTMAN, L. G. KILBORN and LOIS FRASER	0.25
No. 18	Vascular changes produced by adrenalin in vertebrates, by FRANK A. HARTMAN, LESLIE G. KILBORN and ROSS S. LANG	0.25
No. 19	Simplified gas analysis, by J. J. R. MACLEOD	0.25
No. 20	Adrenalin vasodilator mechanisms, by FRANK A. HARTMAN, LESLIE G. KILBORN and LOIS FRASER	0.50
No. 21	Constriction from adrenalin acting upon sympathetic and dorsal root ganglia, by FRANK A. HARTMAN, LESLIE G. KILBORN and LOIS FRASER	
No. 22	The spontaneous development of an acidosis condition in decerebrate cats, by J. J. R. MACLEOD	0.25
No. 23	The diagnosis of acidosis, by J. J. R. MACLEOD	0.25